

Claims

We claim:

- 5           1. An isolated polynucleotide encoding a plant 2-phenylethanol dehydrogenase enzyme, or an enzymatically active fragment thereof.
2. The polynucleotide according to claim 1, wherein said polynucleotide encodes a 2-phenylethanol dehydrogenase of tomato.
- 10           3. The polynucleotide according to claim 2, wherein said 2-phenylethanol dehydrogenase has an amino acid sequence shown in SEQ ID NO: 2.
4. The polynucleotide according to claim 3, wherein said polynucleotide comprises a
- 15   nucleotide sequence shown in SEQ ID NO: 1.
5. The polynucleotide according to claim 1, wherein said polynucleotide encodes a 2-phenylethanol dehydrogenase of petunia.
- 20           6. The polynucleotide according to claim 5, wherein said 2-phenylethanol dehydrogenase has the nucleotide sequence shown in SEQ ID NO: 11.
7. The polynucleotide according to claim 6, wherein said polynucleotide comprises the nucleotide sequence shown in SEQ ID NO: 10.
- 25           8. An isolated polynucleotide encoding a plant phenylalanine decarboxylase, or an enzymatically active fragment thereof.
9. The polynucleotide according to claim 8, wherein said polynucleotide encodes a
- 30   phenylalanine decarboxylase of tomato.
10. The polynucleotide according to claim 9, wherein said phenylalanine decarboxylase has an amino acid sequence shown in SEQ ID NO: 5.

11. The polynucleotide according to claim 9, wherein said phenylalanine decarboxylase has an amino acid sequence shown in SEQ ID NO: 7.

5 12. The polynucleotide according to claim 9, wherein said phenylalanine decarboxylase has an amino acid sequence shown in SEQ ID NO: 9.

13. The polynucleotide according to claim 9, wherein said phenylalanine decarboxylase has an amino acid sequence shown in SEQ ID NO: 13.

10 14. The polynucleotide according to claim 9, wherein said polynucleotide comprises a nucleotide sequence shown in SEQ ID NO: 4.

15 15. The polynucleotide according to claim 9, wherein said polynucleotide comprises a nucleotide sequence shown in SEQ ID NO: 6.

16. The polynucleotide according to claim 9, wherein said polynucleotide comprises a nucleotide sequence shown in SEQ ID NO: 8.

20 17. The polynucleotide according to claim 9, wherein said polynucleotide comprises a nucleotide sequence shown in SEQ ID NO: 12.

18. A polynucleotide expression construct comprising a polynucleotide sequence according to any of claims 1-17.

25 19. The expression construct according to claim 18, wherein said expression construct comprises one or more regulatory elements operably linked to said polynucleotide.

30 20. The expression construct according to claim 19, wherein said regulatory element is selected from the group consisting of a promoter, transcription termination sequence, translation termination sequence, enhancer, and polyadenylation sequence.

21. The expression construct according to claim 20, wherein said promoter is a promoter functional in a plant cell.

22. The expression construct according to claim 21, wherein said promoter is a seed-specific promoter, a tissue-specific promoter, a constitutive promoter, a developmentally-regulated promoter, or an inducible promoter.

23. The expression construct according to claim 22, wherein said seed-specific promoter is a  $\beta$ -phaseolin gene promoter or a glycinin gene promoter.

24. The expression construct according to claim 22, wherein said constitutive promoter is a CaMV promoter, ubiquitin promoter, actin promoter, or NOS promoter.

25. The expression construct according to claim 22, wherein said tissue-specific promoter is a fruit-specific promoter.

26. The expression construct according to claim 22, wherein said fruit-specific promoter is selected from the group consisting of an E8 promoter, a hybrid E4/E8 promoter, an LeExp-1 promoter, and a polygalacturonase- $\beta$  subunit promoter.

27. The expression construct according to claim 22, wherein said tissue-specific promoter is a flower organ-specific promoter.

28. The expression construct according to claim 21, wherein said promoter is selected from the group consisting of a CaMV 35S promoter, CaMV 19S promoter, figwort mosaic virus 35S promoter, *prolifera* promoter, Ap3 promoter, heat shock promoter, T-DNA 1'- or 2'-promoter of *A. tumefaciens*, polygalacturonase promoter, petunia chalcone synthase A (CHS-A) promoter, tobacco PR-1a promoter, ubiquitin promoter, actin promoter, *alcA* gene promoter, *pin2* promoter, maize *WipI* promoter, maize *trpA* gene promoter, maize CDPK gene promoter, and RUBISCO SSU promoter.

29. The expression construct according to claim 18, wherein said expression construct comprises a selectable marker gene.

30. The expression construct according to claim 29, wherein said gene is selected from the group consisting of a gene encoding antibiotic resistance and a gene encoding herbicide resistance.

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31. The expression construct according to claim 30, wherein said antibiotic resistance gene is selected from the group consisting of hygromycin, kanamycin, bleomycin, G418, streptomycin, paromomycin, neomycin, and spectinomycin.

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32. The expression construct according to claim 30, wherein said herbicide resistance gene is a gene that provides resistance to phosphinothricin acetyltransferase or glyphosate.

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33. The expression construct according to claim 29, wherein said gene is selected from the group consisting of genes encoding  $\beta$ -glucuronidase (GUS),  $\beta$ -galactosidase, luciferase, nopaline synthase, chloramphenicol acetyltransferase (CAT), green fluorescence protein (GFP), and enhanced GFP.

34. A cell transformed with a polynucleotide according to any of claims 1-17.

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35. The cell according to claim 34, wherein said polynucleotide is provided in an expression construct according to any of claims 18-33.

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36. A plant, plant tissue, or plant cell transformed with or bred to contain a polynucleotide according to any of claims 1-17.

37. The plant according to claim 36, wherein said polynucleotide is provided in an expression construct.

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38. The plant according to claim 36, wherein said plant is a monocotyledonous plant.

39. The plant according to claim 38, wherein said monocotyledonous plant is selected from the group consisting of rice, wheat, barley, oats, sorghum, maize, sugarcane, pineapple, onion, bananas, coconut, lilies, grasses, and millet.

40. The plant according to claim 36, wherein said plant is a dicotyledonous plant.

41. The plant according to claim 40, wherein said dicotyledonous plant is selected  
5 from the group consisting of tomato, pea, alfalfa, melon, chickpea, chicory, clover, kale,  
lentil, soybean, tobacco, potato, sweet potato, radish, cabbage, rape, grape, sunflower, lettuce,  
cucumber, watermelon, apple, citrus (*e.g.*, orange, lemon, tangerine, grapefruit, lime), pear,  
plum, peach, fig, currant, muskmelon, squash, cherry, sugar beet, tea, strawberry, blackberry,  
blueberry, raspberry, loganberry, rose, chrysanthemum, sweet pepper, eggplant, and cotton.

10 42. The plant according to claim 36, wherein said plant is a tomato.

43. The plant according to claim 36, wherein said plant is an ornamental plant.

15 44. The plant according to claim 43, wherein said ornamental plant is selected from  
the group consisting of roses, petunias, carnations, orchids, tulips, and gardenias.

45. The plant according to claim 36, wherein said plant is a herb plant.

20 46. The plant according to claim 45, wherein said herb plant is selected from the  
group consisting of parsley, sage, rosemary, and thyme.

25 47. A method for providing a plant with increased flavor or fragrance, said method  
comprising incorporating one or more polynucleotides according to any of claims 1-17 and  
expressing the polypeptide encoded by said one or more polynucleotides.

48. The method according to claim 47, wherein said polynucleotide is provided in an  
expression construct.

30 49. The method according to claim 47, wherein said plant is a monocotyledonous  
plant.

50. The method according to claim 49, wherein said monocotyledonous plant is selected from the group consisting of rice, wheat, barley, oats, sorghum, maize, sugarcane, pineapple, onion, bananas, coconut, lilies, grasses, and millet.

5 51. The method according to claim 47, wherein said plant is a dicotyledonous plant.

52. The method according to claim 51, wherein said dicotyledonous plant is selected from the group consisting of tomato, pea, alfalfa, melon, chickpea, chicory, clover, kale, lentil, soybean, tobacco, potato, sweet potato, radish, cabbage, rape, grape, sunflower, lettuce,  
10 cucumber, watermelon, apple, citrus (*e.g.*, orange, lemon, tangerine, grapefruit, lime), pear, plum, peach, fig, currant, muskmelon, squash, cherry, sugar beet, tea, strawberry, blackberry, blueberry, raspberry, loganberry, rose, chrysanthemum, sweet pepper, eggplant, and cotton.

15 53. The method according to claim 47, wherein said plant is a tomato.

54. The method according to claim 47, wherein said plant is an ornamental plant.

55. The method according to claim 54, wherein said ornamental plant is selected from the group consisting of roses, petunias, carnations, orchids, tulips, and gardenias.

20 56. The method according to claim 47, wherein said plant is a herb plant.

57. The method according to claim 56, wherein said herb plant is selected from the group consisting of parsley, sage, rosemary, and thyme.

25 58. A transgenic plant, plant tissue, or plant cell, wherein said plant, plant tissue or plant cell comprises a polynucleotide according to any of claims 1-17 incorporated in the genome of said plant, plant tissue, or plant cell.

30 59. The plant according to claim 58, wherein said plant is a monocotyledonous plant.

60. The plant according to claim 59, wherein said monocotyledonous plant is selected from the group consisting of rice, wheat, barley, oats, sorghum, maize, sugarcane, pineapple, onion, bananas, coconut, lilies, grasses, and millet.

5 61. The plant according to claim 58, wherein said plant is a dicotyledonous plant.

62. The plant according to claim 61, wherein said dicotyledonous plant is selected from the group consisting of tomato, pea, alfalfa, melon, chickpea, chicory, clover, kale, lentil, soybean, tobacco, potato, sweet potato, radish, cabbage, rape, grape, sunflower, lettuce,  
10 cucumber, watermelon, apple, citrus (e.g., orange, lemon, tangerine, grapefruit, lime), pear, plum, peach, fig, currant, muskmelon, squash, cherry, sugar beet, tea, strawberry, blackberry, blueberry, raspberry, loganberry, rose, chrysanthemum, sweet pepper, eggplant, and cotton.

63. The plant according to claim 58, wherein said plant is a tomato.

15 64. The plant according to claim 58, wherein said plant is an ornamental plant.

65. The plant according to claim 64, wherein said ornamental plant is selected from the group consisting of roses, petunias, carnations, orchids, tulips, and gardenias.

20 66. The plant according to claim 58, wherein said plant is a herb plant.

67. The plant according to claim 66, wherein said herb plant is selected from the group consisting of parsley, sage, rosemary, and thyme.

25 68. An isolated plant 2-phenylethanol dehydrogenase enzyme, or an enzymatically active fragment thereof.

69. The plant 2-phenylethanol dehydrogenase enzyme according to claim 68, wherein  
30 said 2-phenylethanol dehydrogenase enzyme is a 2-phenylethanol dehydrogenase of tomato.

70. The plant 2-phenylethanol dehydrogenase enzyme according to claim 68, wherein said 2-phenylethanol dehydrogenase enzyme has an amino acid sequence shown in SEQ ID NO: 2, or an enzymatically active fragment of SEQ ID NO: 2.

5 71. The plant 2-phenylethanol dehydrogenase enzyme according to claim 68, wherein said 2-phenylethanol dehydrogenase enzyme is 2-phenylethanol dehydrogenase of petunia.

72. The plant 2-phenylethanol dehydrogenase enzyme according to claim 68, wherein said 2-phenylethanol dehydrogenase enzyme has an amino acid sequence shown in SEQ ID  
10 NO: 11, or an enzymatically active fragment of SEQ ID NO: 11.

73. An isolated plant phenylalanine decarboxylase enzyme, or an enzymatically active fragment thereof.

15 74. The plant phenylalanine decarboxylase enzyme according to claim 73, wherein said phenylalanine decarboxylase enzyme is a phenylalanine decarboxylase of tomato.

75. The plant phenylalanine decarboxylase enzyme according to claim 73, wherein said phenylalanine decarboxylase enzyme has an amino acid sequence as shown in any of  
20 SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, or SEQ ID NO: 13, or an enzymatically active fragment of SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, or SEQ ID NO: 13.

76. An oligonucleotide probe or primer that can hybridize to a coding or noncoding sequence of a polynucleotide according to any of claims 1-17.

25 77. The oligonucleotide according to claim 76, wherein said oligonucleotide comprises the nucleotide sequence shown in SEQ ID NO: 3, or a functional fragment thereof.

78. A method for producing 2-phenylethanol, said method comprising contacting a  
30 suitable substrate with a 2-phenylethanol dehydrogenase according to any of claims 68 to 72, wherein said substrate is converted to 2-phenylethanol.

79. The method according to claim 78, wherein said substrate is phenylacetaldehyde.



80. A method for producing phenethylamine, said method comprising contacting a suitable substrate with a phenylalanine decarboxylase according to any of claims 73 to 75, wherein said substrate is converted to phenethylamine.

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81. The method according to claim 80, wherein said substrate is phenylalanine.

82. A method for producing a plant that exhibits increased flavor or fragrance, said method comprising introducing a polynucleotide according to any of claims 1-17 into a plant cell and growing a plant from said plant cell.

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83. The method according to claim 82, wherein said plant grown from said plant cell is selected for expression of said polynucleotide.

84. The method according to claim 82, wherein said polynucleotide is provided in an expression construct according to any of claims 18-33.

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85. The method according to claim 82, wherein said plant is a monocotyledonous plant.

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86. The method according to claim 85, wherein said monocotyledonous plant is selected from the group consisting of rice, wheat, barley, oats, sorghum, maize, sugarcane, pineapple, onion, bananas, coconut, lilies, grasses, and millet.

87. The method according to claim 82, wherein said plant is a dicotyledonous plant.

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88. The method according to claim 87, wherein said dicotyledonous plant is selected from the group consisting of tomato, pea, alfalfa, melon, chickpea, chicory, clover, kale, lentil, soybean, tobacco, potato, sweet potato, radish, cabbage, rape, grape, sunflower, lettuce, cucumber, watermelon, apple, citrus (e.g., orange, lemon, tangerine, grapefruit, lime), pear, plum, peach, fig, currant, muskmelon, squash, cherry, sugar beet, tea, strawberry, blackberry, blueberry, raspberry, loganberry, rose, chrysanthemum, sweet pepper, eggplant, and cotton.

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89. The method according to claim 82, wherein said plant is a tomato.

90. The method according to claim 82, wherein said plant is an ornamental plant.

5        91. The method according to claim 90, wherein said ornamental plant is selected from the group consisting of roses, petunias, carnations, orchids, tulips, and gardenias.

92. The method according to claim 82, wherein said plant is a herb plant.

10       93. The method according to claim 92, wherein said herb plant is selected from the group consisting of parsley, sage, rosemary, and thyme.